two mutations, or one deletion and one mutation in the E1 and E4 early gene regions, wherein the open reading frame 4 of the E4 early gene region is neither deleted nor mutated, and wherein said recombinant adenovirus genome additionally contains a transgene that replaces any one of said deletions.

39. (new) A packaging cell line that supports the growth of a replication defective recombinant adenovirus that carries a deletion of adenovirus rep gene region and is free of helper adenovirus, comprising a cell line that supplies the functions of the E1 and E4 early regions, virus-associated RNA sequences, and adenovirus rep gene region. --

REMARKS

The Examiner has restricted the claims to four distinct inventions. Applicants reaffirm the provisional election with traverse made during a phone conversation on March 7, 1996, to prosecute the subject matter of Group I, Claims 1-2, 6-12, 19-22, 24, and 35-36. Claims 3-5, 13-18, 23, 25-34 and 33-34, covering the non-elected subject matter, are canceled without prejudice to the Applicants' right to prosecute these claims in another application.

1. The Invention

Human adenoviruses have been developed as vehicles for *in vivo* gene delivery of transgenes for human gene therapy. In order for adenoviruses to be a safe vehicle for human gene therapy, the cytopathic effect of infectious adenovirus particles must be eliminated by rendering the virus incapable

of replication. Currently available adenoviruses have a deletion in the viral early gene region 1 (E1). E1 is deleted in an attempt to render the recombinant adenovirus replication defective and therefore incapable of producing infectious viral particles in the subsequently infected target cells. However these recombinant adenoviruses have very serious drawbacks, including: (1) recent studies show that E1 deleted adenoviruses are not completely replication incompetent; (2) the resulting accumulation of late adenovirus proteins is cytotoxic to the target cell and induces an inflammatory response and the destruction of the infected tissue which received the virus; (3) the host immune reponse and the cytotoxic effects prevent long term expression of transgenes introduced by the adenovirus.

The present invention relates to novel recombinant adenoviruses characterized by at least two lethal deletions in early gene regions and the novel packaging cell lines that function to propagate these replication deficient adenoviruses. The recombinant adenoviruses of the present invention address the drawbacks with the currently available adenoviruses, i.e., that these viruses are limited by their lack of persistence and associated host inflammatory response. The present invention provides for second and third generation recombinant adenoviruses harboring at least two lethal deletions, in particular in the E1 and E4 regions. E4 functions in the regulation of host cell protein synthesis and is toxic to cells. The E4 promoter is transactivated by E1, therefore cell lines which contain both E1 and E4 express toxic levels of E4.

The deletion of two essential regions, both the E1 and E4 regions, dramatically minimizes or eliminates the pathogenic effects of direct cytotoxicity to the targeted cells and inflammatory responses in the human body. The resulting virus, however, is replication defective and requires the E1 and E4 functions *in trans* in order to replicate. However, since the expression of E1 activates the expression of E4 which is cytotoxic, no one has been able to develop a cell line that expresses and provides these functions to support viral replication and packaging.

The present invention provides a novel packaging cell line which complements functions of E1 and E4, and optionally the E3 DNA regions. The present invention overcomes the difficulty of establishing a cell line to complement the E1 and E4 functions deleted from the recombinant adenoviruses of the present invention by providing a 293 host cell which contains the E1a, E1b, E2a and E4 gene regions. The E4 gene region has been introduced into 293 cells and placed under the control of an inducible promoter, *e.g.*, a tetracycline inducible promoter, so that in the uninduced state, expression is low enough to avoid toxicity to the host cell, but in the presence of tetracycline is sufficiently activated to make enough E4 gene product to complement the E4 deleted region during virus production.

2. The Rejections Under 35 U.S.C. §112 Should Be Withdrawn

The specification is objected to and all the claims (Claims 1-2, 6-12, 19-22, 24, 29-32, and 35-36) are rejected under 35 U.S.C. §112 for lack of enablement.

The Examiner asserts that the specification fails to enable the use of a cAMP response element binding (CREB) protein promoter to drive expression of an adenoviral protein and that sequences, or directions to obtain sequences, are not provided in the specification. The Examiner's attention is invited to page 10 of the specification, lines 28 to 35, which describes promoters chosen from the CREB regulated gene family such as α-inhibin, β-inhibin, α-gonadotropin, cytochrome c, cytochrome c oxidase complex, glucagon etc., listed in Table I on page 15695 in Kim et al., J. Biol. Chem. 268: 15689-15695, which is incorporated into the specification by reference in its entirety. The specification further describes the 8 bp palindromic sequence which constitutes the consensus cAMP responsive element which is responsible for the inductory effect.

Further, the specification teaches by way of Example, the use of the mouse alpha inhibin promoter, a CREB protein responsive promoter, to drive the expression of the adenoviral E4 early gene region. The Examiner's attention is invited to page 24 lines 5 to 27 which describes the construction of the plasmid which contains the adenoviral E4 early gene region, deleted of its promoter, under the control the CREB responsive promoter, the alpha inhibin promoter. The next Example describes the transfection of 293 cells with this plasmid and the selection of cells expressing the adenoviral E4 protein.

The relatedness of the nucleic acid sequences encoding CREB sites in various cAMP-responsive promoters is well known to those skilled in the art (see Borrelli et al. 1992, Critical Reviews in Oncogenesis 3(4): 321-338). Therefore, given this disclosure describing the homology between the members of the CREB related gene family, one of ordinary skill in the art would be able to select a promoter from the CREB family and express an adenoviral protein regulated by that promoter and have a high expectation that such an approach would be successful.

The Examiner also asserts that the specification fails to enable the use of a transcycline responsive promoter to drive the expression of an adenoviral protein. The claims have been amended to obviate this rejection, specifically Claim 9 has been amended to recite that the inducible promoter is selected from the gene encoding the tetracycline responsive promoter" as opposed to the transcycline promoter. The Examiner's attention is invited to page 15 of the specification, lines 13 to 18, which describes the use of a tetracycline responsive promoter to regulate the expression of an adenoviral protein.

The Examiner contends that the specification does not enable any and all combinations of deletions or mutations in the E1, E3 and E4 regions of an adenovirus. According to the Examiner, Applicants have only demonstrated that adenovirus containing deletions in the E1 region combined with deletions in either the E3 or E4 region can be made using the disclosed techniques. This rejection is in error and should be withdrawn. The Examiner's rejection appears to be based on

the erroneous premise that the specification must predict all combinations.

The issue of enablement in an art that is unpredictable was addressed by the Federal Circuit predecessor court. In re Angstadt, 190 USPQ 214 (C.C.P.A. 1976). In Angstadt, the Applicants were not required to disclose every species that worked, or, for that matter, every species that did not work. The court held that the claims were enabled if one skilled in the art could make and use the combination by following the specification for directions on how to do so.

Analogously, in the instant case, Applicants claim a recombinant adenovirus which contains at least two deletions, two mutations or one deletion and one mutation selected from the group containing E1, E2A, E4 early gene regions, and optionally a deletion of the E3 gene region. The specification includes the methods required to test the induction of the mutant viral production in cell lines. According to Angstadt, the Applicants are not required to disclose every combination that worked, nor every combination, that did not work.

The specification in the instant case contains a recitation of the methods necessary for constructing and testing the combinations, Examples 1-11. Parallel to Angstadt, these methods are not complicated. The recombinant techniques of producing different combination of mutations are readily available to the skilled artisan. Similar to Angstadt, one skilled in the art would merely have to substitute one mutation for another. Thus the court concluded in Angstadt, as the P.T.O. must here, that persons with

ordinary skills in the art, provided with the disclosure, would be able to construct and test the combination of mutations within the scope of the claims.

For the reasons enumerated above, the Applicants assert that the claims are fully enabled and request that the rejections under 35 U.S.C. §112, first paragraph, be withdrawn.

Claims 1-2, 6-9, 11-12, and 31 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The claims have been amended to more particularly point out the invention as claimed. In particular, Claim 1 has been amended to clarify that by gene region "a region of the adenoviral genome containing a gene encoding a cytotoxic protein" is intended. Claim 6 has been amended as suggested by the Examiner to clarify that "a promoter" is intended. Claim 31 has been amended to delete the term "slow-replicating". Therefore, the amended claims are not indefinite within the meaning of 35 U.S.C. §112, second paragraph, and the rejection should be withdrawn.

3. The Rejections Under 35 U.S.C. § 102 Should Be Withdrawn

Claims 11-12, 19-22, 24, 29-31, and 35-36 covering a novel replication defective adenovirus, novel recombinant adenoviruses defective in replication, novel packaging cell lines to support the growth of these viruses and a method of infecting mammalian target cells with these viruses containing a transgene, are rejected under 35 U.S.C. §102(a), as being anticipated by Engelhardt. The Examiner has also rejected

Claims 19-22 and 24 under 35 U.S.C. § 102(a) as being anticipated by Armentano et al (1994). Applicants submit that these rejections are in error and should be withdrawn for the reasons explained below.

The legal test for anticipation under 35 U.S.C. §102 requires that each and every element of the claimed invention be disclosed in a prior art reference in a manner sufficient to enable one skilled in the art to reduce the invention to practice, thus placing the public in possession of the invention. W.L. Gore Associates v. Galock, Inc., 721 F.2d 1540, 1554 (Fed. Cir. 1983) cert. denied 469 U.S. 851 (1984); In re Donohue, 766 F. 2d 531 (Fed. Cir. 1985). Anticipation under 35 U.S.C. §102 requires identity of invention. Scripps Clinic & Research Fdn. v. Genentech Inc., 927 F.2d 1565 (Fed. Cir. 1991).

In this instance, the invention defined by the claims covers novel replication-defective recombinant adenoviruses containing at least two lethal deletions in the early gene regions, in addition to novel packaging cell lines which supply the E1 functions as well as the cytotoxic E4 functions needed to support propagation of these replication-defective adenoviruses.

The cited reference, Engelhardt, describes replication-defective adenoviruses containing a deletion in the E1 region, which is replaced by a transgene, and a temperature sensitive (ts) mutation with E2A DNA binding protein (DBP) coding region. At nonpermissive temperatures, this recombinant virus failed to express late gene products in vitro. However, the ts DBP mutation does not assure a complete blockage of the

viral late gene expression when the resulting recombinant virus is used *in vivo*, and thus, is not truly a lethal mutation.

The adenovirus described in Engelhardt only contains one lethal deletion at the permissive temperature, which is in the E1 region and does not contain a deletion in the E4 early region. Therefore, E1 is the only function that needs to be provided *in trans* for replication, so that 293 cells may be used to package the Engelhardt virus. Engelhardt thus, does not teach novel packaging cell lines.

Englehardt recognizes the problem with currently available recombinant adenoviruses, *i.e.*, that "the application of these viruses has been limited by their lack of persistence and associated host inflammatory response." However, Engelhardt's system is not capable of giving rise to a fully inactive replication-defective virus *in vivo*.

The present invention provides a recombinant adenovirus, with not just one, but *two* lethal deletions which results in the complete block of late gene expression in vivo due to (1) a failure to accumulate late viral transcripts; (2) a reduction in viral late protein synthesis; and therefore, (3) defects in viral particle assembly. The dual deletion of E1 and E4 gene regions dramatically minimizes or eliminates the pathogenic effects of direct cytotoxicity to the targeted cells and inflammatory responses in the body.

The cited reference, Armentano, describes a recombinant adenovirus containing deletions in the E1, E3 and E4 regions in order to enhance the cloning capacity of the current adenoviruses. Armentano does not describe or use a lethal

deletion of E4, but rather deletes only the non-essential E4 open reading frames, so that the adenoviruses described still expresses the E4 functions required for normal DNA replication, late protein synthesis and virus production in vitro. The adenoviruses described in Armentano contain only one lethal deletion, i.e., E1, unlike the recombinant adenoviruses of the present invention which contain two lethal mutations, i.e., E1 and E4. Since Armentano only deletes non-essential regions of the E4, it is not necessary to provide E4 functions in trans for packaging of this virus. Therefore, wild type 293 cells may be used to package the Armentano virus.

Since the E4 function has not been deleted from the virus described in Armentano, it does not ensure a complete block in virus replication, therefore the adenoviruses described in Armentano do not overcome the drawbacks associated with the currently available adenovirus systems. Armentano fails to even realize the serious limitations of the currently available adenoviruses which are overcome by the improved viruses of the present invention, including the fact that deletion of only the E1 functions still results in a low degree of virus replication, leading to an incomplete blockage of viral late gene expression. The host immune responses against the adenoviral late gene products cause inflammation and destruction of infected tissues. In addition, the host immune responses and cytotoxic effects combined prevent a long-term gene expression and also cause a reduced level of gene expression following subsequent administration of adenoviruses.

Anticipation requires that all of the elements and limitations of the claim are found within a single prior art reference. There must be no differences between the claimed invention and the reference disclosure as viewed by one of ordinary skill in the field of the invention. Clearly, for the reasons detailed above, the replication-defective adenoviruses of the present invention differ significantly from the adenoviruses described in Engelhardt and Armentano. Thus, the claims are not anticipated by the cited art and, therefore, the rejections under 35 U.S.C. §102 should be withdrawn.

4. The Rejections Under 35 U.S.C. 103 Should Be Withdrawn

All claims (Claims 1-2, 6-12, 19-22, 24, 29-32, and 35-36) covering a novel replication defective adenovirus, novel recombinant adenoviruses defective in replication, novel packaging cell lines to support the growth of these viruses and a method of infecting mammalian target cells with these viruss containing a transgene, are rejected under 35 U.S.C. §103 as obvious over Weinberg, Gregory, Su and Pei.

Briefly, the Examiner contends that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the plasmid described by Weinberg, containing the promoter of Su and Pei, to stably transfect 293 cells thereby allowing for the production of E1/E4-deleted adenoviruses of the present invention. The Examiner also contends that Gregory teaches that a cell line which

complements both an E1 and E4 deletion in an adenoviruses could be established.

A finding of obviousness under § 103 requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere 383 U.S. 1 (1966). The relevant inquiry is whether the prior art suggests the invention, and whether the prior art provides one of ordinary skill in the art with a reasonable expectation of success. In re O'Farrell 853 F.2d 894, 903 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art and not in the Applicants' disclosure. In re Vaeck 947 F.2d 488 (Fed. Cir. 1991).

In the present instance, the relevant inquiry is, first, whether the cited art suggests (1) that the deletion of both E1 and E4 is required to completely block replication of adenovirus, and (2) how to engineer a "non-suicidal" packaging cell line which supplies both the E1 and E4 functions. Moreover, assumimg arguendo that the prior art provided such a suggestion, the second inquiry is whether it provides one of ordinary skill in the art with a reasonable expectation of success. In re O'Farrell; In re Vaeck, supra. Applicants assert, however, that the prior art neither suggests the adenoviruses of the present invention containing two lethal deletions and the cell lines of present invention which

provide these functions, nor gives any reasonable expectation of success.

The Examiner's rejection relies on his contention that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the plasmid described by Weinberg to stably transfect 293 cells thereby allowing for the production of E1/E4-deleted adenoviruses.

The Examiner's contention is based on several erroneous assumptions. First, the art simply does not provide the suggestion of deleting both E1 and E4. Not one of the references cited recognizes the importance of deleting both E1 and E4 in order to completely block adenovirus replication. The references recognize an advantage of deleting non-essential open reading frames of adenovirus in order to create more room to insert transgenes, but not the importance of deleting essential regions to completely block virus replication and the pathogenic effects. In fact, Weinberg's choice of Vero cells which do not complement E1 mutations pointedly excludes the use of E1/E4-deleted vectors.

The Examiner's rejection further relies on the contention that Gregory teaches that a cell line could be established which complements both an E1 and E4 deletion in an adenoviral vetor. The Examiner's contention is in error.

Gregory does not teach methods for producing adenoviruses with two lethal deletions. The adenoviruses disclosed in Gregory contain deletions in E1 or E3 in combination with deletions of the non-essential open reading frames of E4. Gregory suggests deleting non-essential regions of E4 in order to enhance cloning capacity, but retains the essential region

of E4 in order to maintain E4 functions in the virus. Gregory does not teach the use of adenoviruses with deletions of the essential regions of E1 and E4 because it was not known how to provide both of these functions in a packaging cell line.

The passage that the Examiner specifically refers to in Gregory on page 51 is basically a wish list on the part of Gregory. Gregory would like to be able to delete more nucleotide sequences in the E4 region in order to further enhance cloning capacity, but can not, because it was not known how to supply both E1 and E4 functions in a "non-suicidal" packaging cell line. Gregory recognizes the problem with establishing a cell line that would provide both of these functions, i.e., the E4 promoter is activated by the E1a gene product and E4 is toxic to the cell. Gregory speculates the transcription of E4 might be controlled by a transactivating system, but he provides no suggestion or guidance of how to accomplish this goal. Therefore, Gregory and Weinberg, either alone or in combination, do not provide the suggestion of creating an adenovirus containing two lethal deletions, nor do they solve the problem of establishing a packaging cell line which provides the deleted functions.

There is no motivation to combine Su and Pei with Gregory and Weinberg. Su and Pei describe the characterization of the gene sequence of the inducible promoter of the neurine inhibin α gene, but there is no suggestion that this promoter could be used to regulate the transcription of the cytotoxic protein E4 in 293 cells. The Examiner states that one would have been motivated to use the promoter of Su and Pei since it was well known at the time that promoters containing cAMP responsive

elements inducibly regulated gene expression. However there were many inducible promoters known at the time. Therefore the suggestion lacking in Weinberg and Gregory is certainly not provided by Su and Pei.

In view of the foregoing, the art relied on by the Examiner does not render obvious the replication-defective adenoviruses and packaging cell lines of the claimed invention.

CONCLUSION

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. The claims are believed to be free of the art, and patentable. Withdrawal of all the rejections and objections and an early allowance is earnestly sought.

Respectfully submitted,

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